

Urine Collection Protocol for Exome Analysis

1. Urine Collection Protocol for Exosome Analysis

1. Collect urine from PD and controls. Inform participants to collect as much as possible into cup (250cc or larger container)(example of a product that has worked well in our clinic):
 - Globe Scientific 6.5 oz Collection Cup with Pour Spout, Polypropylene (PP), Cup with Paper Lids, 25/Pack, 20 Packs/Case 5924
 - Within a few minutes of participant delivering the specimen, distribute the urine from the pour cup into pre-made 50 mL polypropylene centrifuge tubes (Falcon or Corning, **rated for 10kG spins**, that contain 2 mL of a 20X additive (1M Tris pH7.4, 40mM EGTA). Add 38 mL of urine to each tube, and invert several times to mix. Tubes should be labeled with GUID and date of collection.
 - Collect all of the sample and after collection, place tubes as soon as possible (within an hour) into 4°C for up to 24 hours, -20°C for up to 24 hours, or -80°C indefinitely (samples do not need to be placed at -80°C immediately). Pre-centrifugation is not necessary and this can be done after a rapid thawing protocol
2. *For specimens less than 38mls, add as much as remainder allows, but discard < 20mL of aliquot.*
3. For all samples (after rapid thaw procedure for tubes held at -20°C or -80°C, or within 2-24 hours at 4°C, using a **Beckman J2-HS Centrifuge (or comparable)**, spin down the samples for 15-minutes @ 10,000-rpm (~10,000 kG) / 4°C using the **JA-12 Rotor (or comparable)**. If no centrifuge is available, a more expensive and cumbersome Millipore Steriflip 0.22um filter can be used if there is access to a vacuum line.
4. After spin, slowly decant supernatant into a clean 50mL tube. Observe the left over pellet composition and coloration before discarding. Make note of pellets with unusual characteristics such as red/brown coloration directly on 50 mL tube. Discard original tube with pellet.
5. Immediately freeze supernatant in new 50 mL tube by placing for storage upright into a -80C freezer. After freezing, tube can be placed on its side. 50mL tube needs to have a -80C resistant label/bar code indicator that will not fall off during the thawing water bath process. It is best to also directly label tube with chemical resistant marker in addition to a sticker. Samples may be shipped on dry ice.